Creosote P2 blend: Developmental Toxicity in Rats
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Data Evaluation Record

DEVELOPMENTAL TOXICITY STUDY IN RATS: North American P2 Creosote.
Raymond G. York (March 10, 1995), IRDC, 500 North Main Street, Mattawan, MI, Report number 671-022, The Creosote Council II, Mellon Hall, Duquesne University, Pittsburgh, U.S.A. MRID # 43584202. Unpublished.

Note: The in-life phase of the study was performed from July 16, 1993 to August 19, 1993. The study was conducted in compliance with the United States Environmental Protection Agency Federal Insecticide, Fungicide and Rodenticide Act Good Laboratory Practice Standards.

TEST MATERIAL:

The active ingredient was P2 Creosote (Pro. No. TOR-236705-0, H. A. Kremer, Bolton, ON), a black liquid (composite mixture), stable at room temp. for the duration of the study. Corn oil (Lot No. 1063.04, Bio-Serv, A Holton Industries Co., NJ) was used as vehicle control. Individual dosages were determined from the most recently recorded individual body weights.

Test articles were prepared by heating the test article to 40^{0}C, weighing the required amount and mixing it with corn oil (vehicle) in order to permit the administration of dose levels of 25, 75 and 225 mg/kg bw/day at a volume of 10 ml/kg bw/day. Samples were within 94-100% of the desired concentration of Creosote. Creosote was homogenously distributed in the dosage formulations (25, 75 and 225 mg/kg bw/day dose levels had relative standard deviations of 3.3, 1.7 and 2.8% respectively) and was stable for at least 10 days at room temperature.

TEST ANIMALS:

Sprague-Dawley rats (Crl:CD VAF/Plus) (Charles River Laboratories, Michigan), 77-day old, sexually mature, virgin females, 212-300 g (at beginning of study).

DOSE LEVELS: 0, 25, 75 and 225 mg/kg bw/day in a 10 ml/kg bw/day volume by gavage. 30 rats/dose level.

ANIMAL HUSBANDRY:

The animals were housed individually in stainless steel, wire mesh cages, maintained at 21-22^{0}C, 55-60 % relative humidity and a 12-hour photocycle, acclimated for 10 days prior to experimentation. Food [a premix of laboratory diet (Certified Rodent Chow # 5002; Purina Mills, Inc., St. Louis, Missouri)] and water were available *ad libitum*.

METHOD:

Study Design:

The dose selection was based on the results of a range finding developmental toxicity study (study no. 671-021) performed at dose levels of 0, 30, 60, 180, 325, 600, 1200 and 1800 mg/kg bw/day of P2 Creosote by gavage. In this study maternal toxicity was manifested as mortalities (1200 and 1800 mg/kg bw/day), toxic clinical signs and decreased body weight gains (325 and 600 mg/kg bw/day). No evidence of maternal or developmental toxicity was observed at the 30 or 60 mg/kg bw/day dose level.

After the acclimation period, one male and one female were caged together for mating. Upon evidence of copulation (day the copulatory plug was observed, gestation day-0), the female was returned to an individual cage. Mated female rats were distributed into four dose groups. The control animals received corn oil and the test animals received prepared Creosote by oral gavage on gestation days 6 through 15. On gestation day-20, all surviving animals were euthanized by carbon dioxide inhalation, followed immediately by Cesarean section examination.

Maternal Observations:

General Observation: All animals were observed for mortality and morbidity (twice daily), clinical signs (daily), body weight measurement (gestation days 0, 6, 9, 12, 16 and 20) and measurement of food consumption (gestation days 0, 6, 9, 12, 16 and 20).

Reproductive Performance: For each animal, the data recorded on gestation day-20 by Cesarean section examination included: number of females pregnant, demonstrated by the presence of implantation sites observed at autopsy, number of animals with viable fetuses, number of animals with complete resorptions, early (implantation site, tissue has no recognizable fetal characteristics) and late (recognizable fetal form, but undergoing autolysis) resorptions, numbers of total implantations and corpora lutea, uterine weight, location of viable and non-viable fetuses, abdominal and thoracic cavities and gross morphological changes of organs.

Litter and Fetal Observations:

Data recorded included: fetal number, weight and sex distribution, crown-rump lengths and all gross, visceral (Wilson razor-blade sectioning method) and skeletal (Dawson method) alterations, which were classified as malformations or developmental variations.

STATISTICS:

Analysis of variance, Bartletts test for homogeneity of variance, Student's t-test, Chi-square test, Fisher's exact test, Mann-Whitney U-test were carried out to examine all data.

RESULTS:

Maternal Observations:

Mortality: There were no treatment-related deaths during the treatment period. One animal of the high-dose group died on day-14 which was attributed to intubation error.

Clinical Signs: No treatment-related toxic effect was observed.

Body weights: At the 225 mg/kg bw/day dose level, significant reduction in body weight gain when compared to controls was observed during the first dosing sub-interval (gestation days 6-9; 55%), second dosing sub-interval (gestation days 9-12; 68%, $p<0.01$), third dosing sub-interval (gestation days 12-16; 23%), test article administration period (gestation days 6-16; 44%, $p<0.01$), post-dosing period (gestation days 16-20; 25%, $p<0.05$) as well as over the entire gestation period (gestation days 0-20; 24%, $p<0.01$).

At the 75 mg/kg bw/day dose level reduction in body weight gain when compared to controls was seen during the first dosing sub-interval (gestation days 6-9; 39%), second dosing sub-interval (gestation days 9-12; 58%, $p<0.05$) and during the test article administration period (gestation days 6-16; 17%, $p<0.05$). Body weight gains were similar to controls during the post-treatment period (gestation days 16-20) as well as during the entire gestation period (gestation days 0-20).

Table: Mean body weight gains (g) in rats.

Days of Gestation:	Mean Body Weight Gains (g)*						
	0-6 d	6-9 d	9-12 d	12-16 d	16-20 d	6-16 d	0-20 d
Control	30	18	19	34	63	71	164
25 mg/kg bw/day	31	**11**	**12**	32	60	**55***	147
75 mg/kg bw/day	34	**11**	**8***	39	65	**59***	158
225 mg/kg bw/day	37	**8**	**6****	**26**	**47***	**40****	**124****

* $p<0.05$, ** $p<0.01$.

At the 25 mg/kg bw/day dose level reduced body weight gain when compared to controls was seen during the first dosing sub-interval (gestation days 6-9; 39%), second dosing sub-interval (gestation days 9-12; 37%) and during the test article administration period (gestation days 6-16; 22%, $p<0.05$). Body weight gains were similar to controls during the post-treatment period (gestation days 16-20) as well as during the entire gestation period (gestation days 0-20).

These findings correspond to reduced food-consumption values and are considered treatment-related toxicologically significant effects and indicate maternal toxicity at all dose levels.

Food Consumption: Treatment-related significant reductions in food consumption when compared to controls was observed (following Table) during the treatment period at all dose levels. In the low, mid and high-dose groups, reduction in food consumption was seen during the gestation day intervals 6-9 (14%, $p<0.05$; 22%, $p<0.01$ and 27%, $p<0.01$ respectively), 9-12 (17%, $p<0.01$; 13% and 23%, $p<0.05$ respectively), during the test article administration period

i.e. gestation days 6-16 (15%, $p<0.05$; 13%, $p<0.01$ and 21%, $p<0.01$ respectively) as well as over the entire gestation period i.e. gestation days 0-20 (9%, $p<0.01$; 7%, $p<0.01$ and 10%, $p<0.01$ respectively) when compared to controls. Reduction in food consumption during the dosing period at all dose levels corresponded to reduced body weight gain and were considered to be a treatment-related toxicologically significant effect. Food consumption values during all other measured intervals were similar to those for control dams. After cessation of dosing (gestation days 16-20), food consumption of all test groups returned to the control values.

Table: Mean food consumption (g/kg bw/day) in rats.

Days of Gestation:	Food Consumption Values (g/animal/day)						
	0-6 d	**6-9 d**	**9-12 d**	**12-16 d**	**16-20 d**	**6-16 d**	**0-20 d**
Control	23.1	21.4	21.6	24.3	28.0	22.6	23.8
25 mg/kg bw/day	21.8	**18.4***	**17.8****	20.6	27.6	**19.1***	**21.6****
75 mg/kg bw/day	22.4	**16.6****	**18.8**	22.4	28.2	**19.6****	**22.1****
225 mg/kg bw/day	22.7	**15.6****	**16.6****	20.5	28.1	**17.9****	**21.4****

* $p<0.05$, **<0.01.

The overall pattern of food consumption indicates that effects were observed at all test-groups during the period of dosing. Calculation of food efficiency was made for the dosing period (days 6-16) as well as for the study period as a whole. Results are shown below:

	0 mg/kg/day	25 mg/kg/day	75 mg/kg/day	225 mg/kg/day
Study days 6-16	35.3%	30.9%	30.6%	24.3%
Study days 0-20	54.6%	53.2%	52.5%	46.3%

As noted from these data, food efficiency was affected at the high dose for both the period of dosing and for the study period as a whole. The decrease appeared dose-related.

Gross Necropsy Findings: No treatment-related effects were observed at necropsy. The animal of the high-dose group that died showed clots and severely red thoracic cavity as well as discoloured, multilobular, red lung at necropsy, suggesting error during intubation.

Reproductive Data: Treatment-related toxicologically significant effects on the reproductive parameters observed (following Table) at the 225 mg/kg bw/day dose level when compared to controls included: significantly increased post-implantation loss and % post-implantation loss

(>4-fold higher, all early resorption). One whole litter resorption was also observed at this dose level. Reduced gravid uterine weight (36% less) when compared to controls reflects the reduced number of live fetuses and the reduced fetal weight.

At the 75 and 25 mg/kg bw/day dose levels, significantly reduced number of implantation sites and one whole litter resorption at the low-dose level was within the range of historical control values therefore was not considered treatment-related.

Table: Reproductive effects of P2 Creosote by oral administration in pregnant rats.

Observations	Creosote Dose Levels (mg/kg bw/day)			
	0	**25**	**75**	**225**
No. of females mated	30	30	30	30
No. (%) of pregnant females	24 (80%)	20 (67%)	29 (97%)	23 (77%)
No. of dams with viable fetuses	24	19	29	22
No. of dams with complete resorption	0	1	0	1
No. of corpora lutea/dam	18.6	17.3	17.9	17.1
No. of implantation sites/dam	16.8	15.2*	16.1	**14.9**
No. of post-implantation loss/dam	1.1	1.7	1.4	**5.3****
% Pre-implantation loss	9.4	11.0	10.2	**13.3**
% Post-implantation loss	6.7	10.9	8.8	**35.7**
Mean resorption total	1.1	1.7	1.4	**5.3**
Mean uterine weight (g)	87.3	78.7	81.5	**55.9**

* $p < 0.05$, ** $p < 0.01$.

Litter Data:

Treatment-related toxicologically significant effects observed at the 225 mg/kg bw/day dose level includes: significantly reduced number of live fetuses/litter (38%, $p<0.01$) and reduced mean fetal body weight (8%, $p<0.05$) when compared to controls (following Table). There were no treatment-related effects at the 75 mg/kg bw/day dose level. Significantly reduced number of live fetuses/litter (14%, $p<0.05$) at the 25 mg/kg bw/day dose group when compared to controls was within the range of historical controls and therefore was not considered treatment-related.

Table: Litter effects of P1/P13 Creosote by oral administration in pregnant rats.

Litter Observations	Creosote Dosage Levels (mg/kg bw/day)			
	0	25	75	225
No. of litters	24	20	29	23
No. of live fetuses/litter	15.7	13.5*	14.7	**9.7****
No. of dead fetuses	0	0	0	0
No. of male fetuses/litter	8.1	7.9	7.2	5.2
No. of female fetuses/litter	7.6	6.3	7.4	4.9
Mean fetal body weight (g)	3.6	3.6	3.7	**3.3***
Mean fetal crown rump length (cm)	3.6	3.6	3.6	3.5

* $p < 0.05$, ** $p < 0.01$.

Fetal Observations:

Developmental Malformations:

No significant increase in the incidence of developmental malformations were seen in any of the test groups as compared to controls. The incidence of malformations observed in control and treatment groups as well as the incidence of similar malformations found in animals of the historical control group is shown as the number of fetuses and litters (parentheses) in the following Table.

At the 25 mg/kg bw/day dose level, the incidence of malformations were within the range of historical control, and therefore, are not considered treatment-related.

At the 75 mg/kg bw/day dose level, the single incidences of malformations observed as craniorachischisis, hydrocephaly and malpositioned eye were in fetuses from different dams. No such incidence was seen in the low-dose group, control group or in historical controls and therefore, this incidence was considered as treatment-related. Other observations were within the range of historical controls.

At the 225 mg/kg bw/day dose level, a single incidence of hydrocephaly was observed, which was also present at the mid-dose group but not in the low-dose group, control group or in the historical control animals, and therefore, this incidence was considered as treatment-related. (Note: the number of fetuses examined at the high-dose level for visceral and skeletal malformations are approximately 50% of those examined at mid-dose level).

Table: Incidence of fetal malformations observed, shown as number of fetuses (litters).

INCIDENCE	Historical Control	Creosote Dose Level (mg/kg bw/day)			
		0	25	75	225
Total No. of litters examined:	598	24	19	29	22
No. of fetuses examined externally:	8429	377	270	425	222
No. of fetuses examined viscerally:	2429	187	136	212	113
No. of fetuses examined skeletally:	2898	190	134	213	109
Incidence of Malformations					
Cephalic: Microphthalmia	5(5)	0	0	1(1)	0
Craniorachischisis	0	0	0	1(1)	0
Micrognathia	4(4)	0	0	1(1)	0
Folded retina	2(2)	1(1)	1(1)	0	0
Hydrocephaly	0	0	0	1(1)*	1(1)
Anophthalmia	1(1)	0	0	1(1)*	0
Eye malpositioned	0	0	0	1(1)*	0
Gastrointestinal: Omphalocele	4(4)	0	1(1)	0	0
Vertebral column & Ribs:Vertebral malformations	6(6)	0	0	1(1)	0
Interrupted ossification of the vertebral arch:	0	1(1)	0	0	0
Other: Anasarca	1(1)	0	0	1(1)	0

* Malformations from a single fetus.

Developmental Variations:

The major developmental variations that were observed included: distended ureter, renal papillae not developed, hyoid unossified, 14th rudimentary rib, less than 13 pairs of full ribs and sternebra #5 and #6 unossified. These developmental variations were sporadic, not dose-related and were within the range of historical controls. These findings were not considered treatment-related.

AUTHOR'S CONCLUSIONS:

The authors concluded that P2 Creosote administration caused maternal toxicity at all the dose levels tested, developmental toxicity at the 225 mg/kg bw/day dose level and no teratogenic effect at any of the tested dose levels.

Executive Summary:

In a developmental toxicity study (MRID # 43584202), pregnant female Sprague-Dawley rats (30/dose) were administered P2 creosote by gavage on gestation days 6 through 15 inclusive at dose levels of 0, 25, 75, and 225 mg/kg/day.

Although decreases in body weight and food consumption were observed at all dose levels, food efficiency appeared affected only at the high dose of 225 mg/kg/day. Thus, the cesarean section effects observed at this dose (decreased implantations/dam, increased pre- and post-implantation loss, increased resorptions, decreased uterine weight) could be secondary to the decreased food efficiency observed at this dose. This distinction is a fine one, however, based on results with P1/P13 creosote in which no decrease in food efficiency was observed but similar effects at cesarean section were noted. It is quite possible that the P2 blend results in a different spectrum of developmentally toxic effects than the P1/P13 blend. Based on the data in this study, the Maternal NOAEL is determined to be 75 mg/kg/day, based on decreased food efficiency observed at 225 mg/kg/day.

With regards to assessing the teratogenic potential of P2 Creosote in rats, no treatment-related malformations (external, visceral or skeletal) were observed at the 25 mg/kg bw/day dose level. The single incidences of malformations [craniorachischisis, hydrocephaly and malpositioned eye (same pup)] observed at the 75 mg/kg bw/day dose level, and hydrocephaly at the 225 mg/kg bw/day dose level compared to none observed in lower dose levels, concurrent controls or historical controls (2429 and 2898 fetuses examined viscerally and skeletally respectively) were considered treatment-related. It should be noted that at 225 mg/kg bw/day one whole litter was resorbed and the number of fetuses examined at this level for visceral and skeletal malformations was approximately 50% of those examined at the mid-dose level. The developmental toxicity NOAEL is determined to be 25 mg/kg/day in this study, based on the incidences of malformations observed at 75 mg/kg/day which exceeded both concurrent and historical control incidence.

This study is classified as **acceptable** and satisfies the guideline requirement (83-3) for a developmental toxicity study in rats.

Taken together, the results of the two developmental toxicity studies with creosote indicate potential increased sensitivity of developing mammalian organisms to the toxic effects of P1/P13 and P2 creosote. Further study in the form of a developmental neurotoxicity study with each blend is warranted.